

Evolutionary and Population Genetics of *Jasus* Species Inferred from Mitochondrial DNA

Darren John Brasher B.App.Sc. (Bendigo)

A thesis submitted to the Department of Zoology, University of Tasmania, in partial
fulfilment of the requirements for the degree of Master of Science

January 1994

Table of Contents

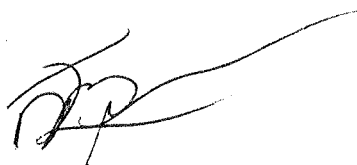
Declaration and Statement	i
Summary	ii
Acknowledgements	iv
1. General Introduction	1
1.1 Molecular Systematics	1
1.2 Rock Lobsters (Genus <i>Jasus</i>)	2
1.2.1 Taxonomy	2
1.2.2 Life History	4
1.3 Genetic Approach to Populations	5
1.3.1 Properties of the Mitochondrial Genome	5
1.3.2 Restriction Enzyme Analysis of mtDNA	7
1.3.2.1 Detection of Population Subdivision	8
1.3.2.2 Phylogenetic Reconstruction of Closely Related Taxa	10
1.3.3 Protein Electrophoresis	13
1.3.4 Comparison of the Resolution of mtDNA and Protein Analyses	14
1.4 Genetic Approach to Management of Exploited Populations	15
1.4.1 Recognition of Genetically Defined Stocks	15
1.4.2 Marine Stock Assessment using mtDNA	16
1.4.3 Protein Analysis of Lobster Species	17
1.5 Background to Commercial <i>Jasus</i> Fisheries	19
1.5.1 Australian Fishery	19
1.5.2 New Zealand Fishery	20
1.6 Aims of the Study	22
2. Materials and Methods	23
2.1 Sample Collection	23
2.2 Isolation of Mitochondria	23
2.3 Isolation of Mitochondrial DNA	24
2.4 Enzymatic Digestion and Identification of mtDNA Fragments	24
2.5 Restriction Site Mapping of mtDNA	26
2.6 Data Analysis	27
2.6.1 Interpretation of mtDNA Fragments	27
2.6.2 Analysis of Nucleotide Sequence Diversity	27

2.6.3 Gene Diversity Analysis	28
2.6.4 Genotypic Diversity Analysis	29
2.6.5 Phylogenetic Reconstruction	29
2.6.5.1 Discrete-character Methods	29
2.6.5.2 Distance Matrix Methods	30
3. Evolutionary Genetics of <i>Jasus</i> Species	31
3.1 Introduction	31
3.2 Results	32
3.2.1 Enzymatic Digestion	32
3.2.2 Intraspecific mtDNA Variation	32
3.2.3 Genetic Similarity of <i>J. novaehollandiae</i> and <i>J. edwardsii</i>	32
3.2.4 Genetically Defined Species	34
3.2.5 Phylogenetic Reconstruction	37
3.2.5.1 <i>J. verreauxi</i> as an Outgroup	37
3.2.5.2 Discrete-character Analysis	37
3.2.5.3 Phenetic Analysis	38
3.2.5.4 Absence of Geographic Partitioning in <i>J. edwardsii</i>	38
3.3 Discussion	38
3.3.1 Support for 'verreauxi' and 'lalandii' Groups	38
3.3.2 Gene Flow Between Australian and New Zealand <i>J. edwardsii</i>	39
3.3.3 High mtDNA Polymorphism in <i>Jasus</i>	40
3.3.4 Validity of Phylogenetic Reconstructions	40
3.3.5 Speciation of <i>J. tristani</i> and <i>J. lalandii</i>	40
3.3.6 Evolution of 'lalandii' Group <i>Jasus</i>	41
3.3.7 Systematic Value of 'lalandii' and 'frontalis' Subgroups	43
3.3.8 MtDNA and Introgressive Hybridization	44
3.3.9 Rate of mtDNA Evolution in <i>Jasus</i>	44
4. Population Genetics of <i>Jasus edwardsii</i>	45
4.1 Introduction	45
4.2 Results	46
4.2.1 Enzymatic Digestion	46
4.2.2 Phenetic Analysis	48
4.2.3 Intra- and Inter-Populational Sequence Diversity	48
4.2.4 Grouping of Locations for Gene Diversity Analyses	54
4.3 Discussion	55
4.3.1 Scenarios for Restrictions to Gene Flow	55
4.3.2 Significance of Inter-Populational Sequence Comparisons	55
4.3.3 Apparent Absence of Population Subdivision	56

4.3.4 Conservation Status of Western Populations	56
5. Population Genetics of <i>Jasus verreauxi</i>	58
5.1 Introduction	58
5.2 Results	59
5.2.1 Enzymatic Digestion	59
5.2.2 Gene Diversity Analysis	62
5.3 Discussion	62
5.3.1 Comparison with <i>J. edwardsii</i> Population Study	62
5.3.2 Factors Promoting Subdivision in <i>J. verreauxi</i>	63
6. Conclusions and General Discussion	66
6.1 Taxonomy of <i>Jasus</i>	66
6.2 Population Structure of <i>J. edwardsii</i> and <i>J. verreauxi</i>	67
6.3 Application of Results to Fishery Management	68
6.4 Speciation in <i>Jasus</i>	70
References	73
Appendices	84
Appendix 3a	84
Appendix 4a	87

Declaration and Statement

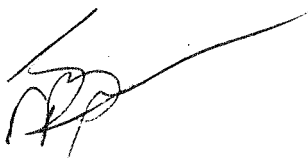
I hereby declare that this thesis contains no material which has been accepted for the award of any degree or diploma in any university and that, to the best of my knowledge, this thesis contains no copy of previously published material except where due reference is made in the text.



D.J. Brasher

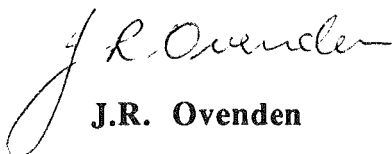
18 January 1994

The work reported in this thesis was carried out by D.J. Brasher under the supervision of Drs R.W.G. White and J.R. Ovenden using funds from a grant to Drs Ovenden and White from the Fishing Industry Research and Development Council (FIRDC). Most of the material in this thesis has already been published; references to the publications are given at the beginning of each of Chapters 3, 4 and 5. Mr. Brasher is the senior author on two of the papers (reported in Chapters 3 and 5); Dr Ovenden is the senior author on the the third paper (reported in Chapter 4). Mr Brasher carried out all of the practical work and the majority of the analyses for this latter paper. Dr Ovenden's seniority in the authorship list recognises her inception of the project, her success in obtaining the research funds and her supervisory and creative input.

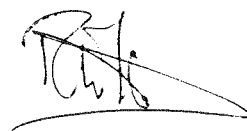


D.J. Brasher

18 January 1994



J.R. Ovenden



R.W.G. White

Summary

Restriction enzyme analysis of mtDNA extracted from antennal gland tissue was used to assess mtDNA variation within and between five nominal species of commercially important rock lobster, genus *Jasus*. Samples were collected from South Africa (*Jasus lalandii*, sample size=11), Vema Seamount in the South Atlantic Ocean (*J. tristani*, 8), Tasmania (*J. novaehollandiae*, 11), and New Zealand (*J. edwardsii*, 10; *J. verreauxi*, 9).

The generally high degree of intraspecific mtDNA nucleotide sequence diversity detected (0.33–0.99%) with six five- and six-base enzymes enabled high resolution assessment of species boundaries previously defined only by morphological criteria. Sequence diversity analysis failed to reveal significant mtDNA diversity between the *J. edwardsii* and *J. novaehollandiae* samples. Furthermore, phylogenetic reconstruction, using both phenetic and parsimony analysis of the restriction site data, did not partition mtDNA haplotypes into distinct Australian and New Zealand assemblages. These results suggest that Australian and New Zealand red rock lobster represent conspecific populations of *J. edwardsii*.

High sequence diversities separating *J. edwardsii*, *J. lalandii* and *J. tristani* (4.41–7.36%) indicates long-term reproductive isolation and provides confirmation of their specific status. The genome of *J. verreauxi* is highly distinct from the genomes of the other species (nucleotide sequence diversity: 14.92–16.67%), supporting the existence of '*lalandii*' and '*verreauxi*' groups within *Jasus*.

The topologies of trees generated by phylogenetic analyses grouped *J. edwardsii* and *J. lalandii* to the exclusion of *J. tristani*; however, further analysis indicated that the relationships of these species was essentially unresolved. Nevertheless, these results, when considered with knowledge of the current distributions of these species, do not support hypotheses for the evolution of '*lalandii*' group *Jasus*, which suggest a relatively recent divergence of *J. lalandii* and *J. tristani*. Instead, they give systematic validity to the grouping of *J. lalandii* with *J. edwardsii* as proposed by the existing taxonomy.

A restriction enzyme study of mtDNA variation in *J. edwardsii* was extended to include samples collected from widespread locales across southern Australia and from New Zealand. A total of 132 adult lobsters from 13 locales were analysed to address the possibility of population subdivision. Phenetic analysis failed to reveal geographic partitioning of mtDNA haplotypes. Despite collection locations being separated by large geographical distances, sequence diversity analysis between pairs of samples failed to conclusively detect population subdivision. Gene diversity analyses also failed to detect subdivision, even when samples were grouped into likely populations based on hydrological and environmental parameters. These results suggest that genetic homogeneity is maintained in this species by widespread dispersal of a long-lived, planktonic larval stage.

Analyses to detect population subdivision were also applied to 25 *J. verreauxi* collected from one Australian and two New Zealand locales. From the raw restriction site data, Australian and New Zealand haplotype assemblages were defined by two restriction sites, one confined to each country. Genetic differentiation between Australian and New Zealand *J. verreauxi* was also supported by gene diversity analysis. In contrast to the population study of *J. edwardsii*, these results suggest that larval exchange between adult populations across the Tasman Sea may be limited.

Acknowledgements

I would like to thank my supervisor Dr R.W.G. White and co-supervisor Dr J.R. Ovenden for their constructive criticisms, assistance, guidance and encouragement throughout the course of this project.

I would also like to thank other members of the Fish Genetics Laboratory – Adam Smolenski, Russel Bywater, Jenny Jarrett, Tom Krasnicki, Jeremy Austin and Jon Waters – for their invaluable advice, assistance and company, Wayne Kelly for technical support, and Barry Rumbold for his efficient help in the purchasing of materials and equipment.

I gratefully acknowledge the following people for providing rock lobster samples: Robert Kennedy (Tasmanian Department of Sea Fisheries), Robert Grove-Jones and Bruce Wallner (South Australian Department of Fisheries), David Molloy (Marine Science Laboratories, Queenscliff, Victoria), Rhys Brown and Eric Barker (Fisheries Department of Western Australia), John Booth, Keith Allen and Allan Gard (Ministry of Agriculture and Fisheries, New Zealand), Mick Murphy (Temma, Tasmania), George Greaves (Esperance, Western Australia), Dave Pollock and Roy Melville-Smith (Sea Fisheries Research Institute, South Africa), and Steve Montgomery (New South Wales Institute of Fisheries).

My thanks also to Bob Kennedy, Bruce Phillips, Dave Pollock and John Booth for comments or reviews relating to drafts of published manuscripts.

I thank the Fishing Industry Research and Development Council (grant 88/41) for financially supporting this study.

Finally, I wish to give my warmest thanks to Alice Valentine for her unfailing friendship and encouragement during the final preparation of this thesis.